

RESIDUE DEPLETION STUDY OF PCDDs AND PCDFs IN DOSED BEEF CATTLE

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Introduction

Analysis of milk in 1991 from individual farms in Bolsover, Derbyshire, revealed a localised source of contamination with PCDD/Fs. Monitoring over the following years has provided information regarding the depletion rates of PCDD/Fs in lactating cows.^{1,2} Very little work has been undertaken on non-lactating cows. Olling *et al*³ undertook a study on three adult cows maintained at relatively stable body weights. Through the analysis of PCDD/Fs in subcutaneous fat biopsy samples, over a 220 day period, the group determined that the mean half-lives of different congeners were between 160 and 280 days. Startin *et al*⁴ used cattle from the contaminated farms in Bolsover to investigate changes in PCDD/Fs concentrations during fattening. Five animals were removed from the source of contamination, fed an essentially PCDD/F free diet, then sequentially slaughtered over a period of 173 days. The authors reported that the results were consistent with half-lives in the 100 to 200 day range.

This report describes the dosing of beef cattle with known concentrations of five PCDD/Fs. They were fattened under normal animal husbandry conditions and sequentially slaughtered. Post-mortem samples were taken of subcutaneous fat, perirenal fat, muscle and liver. In addition, subcutaneous fat biopsy samples were obtained periodically during the study. Samples of the feed pellets and hay fed to the animals during the study were analysed to ensure that they were essentially free from the PCDD/Fs of interest. Samples of the cattle faeces were also analysed.

Materials and Methods

Ten cattle were treated with daily doses of 150 ± 15 ng of five PCDD/F congeners as indicated in Table 1, over a 4 week period. They were subsequently slaughtered as three groups, at 5, 18 and 31 weeks after the first dose (Groups 1, 2 and 3 respectively).

Samples were extracted and cleaned-up using a modification of the method described by Nygren *et al*.⁵ Tissue samples were analysed in batches of 12, with two analytical blanks and a reference meat sample (a single sample of muscle tissue from the study) included in each batch as quality control. All GC-MS results were scrutinised before acceptance.

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1. Concentrations of PCDD/Fs in Selected Tissues, ng/kg fat weight basis

Slaughter Time (weeks)	2,3,7,8-TCDD				1,2,3,7,8-PeCDD				1,2,3,6,7,8-HxCDD				2,3,4,7,8-PeCDF				1,2,3,4,7,8-PeCDF	
	Tissue		Fat		Tissue		Fat		Tissue		Fat		Tissue		Fat		Tissue	
	L	M	S	P	L	M	S	P	L	M	S	P	L	M	S	P	L	
<u>sed</u>																		
<u>nals</u>																		
a	5	560	210	32	45	560	250	29	32	500	140	26	11	740	230	35	34	580
b	5	460	330	56	50	710	220	43	57	670	120	21	43	760	270	44	50	760
c	5	260	210	55	55	290	280	42	49	120	150	18	34	140	270	45	19	100
a	18	46	21	N/A	N/A	110	13	N/A	N/A	58	16	N/A	N/A	110	18	N/A	N/A	73
b	18	37	37	17	12	55	13	14	8.5	50	11	8.6	14	93	16	16	7.8	91
c	18	30	21	15	15	75	21	12	11	74	18	9.5	12	120	20	16	17	140
a	31	33	22	13	16	56	25	11	12	41	16	9.9	9.3	83	28	16	14	110
b	31	16	38	13	16	39	44	11	12	31	20	9.3	12	64	31	14	15	77
c	31	16	13	12	12	33	25	9.2	8.6	93	14	10	7.5	89	15	12	11	78
d	31	58	N/A	13	12	92	N/A	11	8.6	60	N/A	8.3	12	120	N/A	13	11	140
<u>tr</u>																		
<u>ol</u>																		
<u>nals</u>																		
up 0	-1 day	7.4	4.0	0.8	0.8	5.2	4.6	0.9	1.6	2.5	5.4	0.9	0.7	5.5	6.5	0.8	0.9	0.5
up 1	5	6.1	2.6	1.1	1.0	3.2	2.2	1.0	0.6	2.2	2.1	1.0	0.6	3.7	2.9	0.6	0.7	2.6
up 3	31	1.1	1.6	1.0	1.8	0.2	6.8	0.9	0.4	3.7	4.1	0.7	1.2	3.9	2.4	0.8	1.1	6.0
up 3	31	2.7	0.8	0.7	2.0	0.2	7.3	0.7	3.3	0.2	4.9	0.6	2.9	0.3	0.4	0.6	0.8	5.2

: Sample not available

L: Liver,

M: Muscle

S: Subcutaneous P: Peri-renal

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reement between individual data points in these instances was similar to that obtained for the reference meat sample (see under y control, below).

oncentrations of PCDD/Fs in control animals was low compared with the treated animals throughout the study. In the case of > 1 dosed animals, slaughtered five weeks after the start of dosing, the two different depot fat stores (sub-cutaneous fat and nal fat) generally had similar concentrations to the value predicted from earlier studies.^{3,4} However, liver and muscle contained d 10 and 5 times higher concentrations respectively of PCDD/Fs (on a fat basis) than the fat stores. The differences had ved by the time that Groups 2 and 3 animals were slaughtered although liver and muscle concentrations were still typically twice present in fat stores. Preferential accumulation in animal livers following high doses is in line with published results obtained in species, but the higher concentrations found in muscle were not predicted. This fact suggested that the distribution phase was plete and that PCDD/Fs were still associated mainly with circulating blood lipids rather than as an equilibrium with depot fat.

ges in PCDD/F concentration in subcutaneous fat with time, incorporating both post-mortem and biopsy data from different ls, are shown in Figure 1. The figures are corrected for weight gain of the animals during the study. Starting concentrations ess than 5 ng/kg for each congener in each animal. Each curve follows the same form, showing a large increase in concentration sampled one week post-dosing, followed by a rapid decline over the first part of the depletion study with a slower fall in ntration thereafter.

ives for each congener can be estimated from the plots as follows: 2,3,7,8-TCDD (93 days), 1,2,3,7,8-PeCDD (126 days), 5,7,8,-HxCDD (148 days), 2,3,4,7,8-PeCDF (106 days) and 1,2,3,4,7,8-HxCDF (124 days). These values were broadly in line :alculated values in the literature.^{3,4} The high value for 1,2,3,6,7,8-HxCDD was affected by a single data point which was out of ith the general pattern observed.

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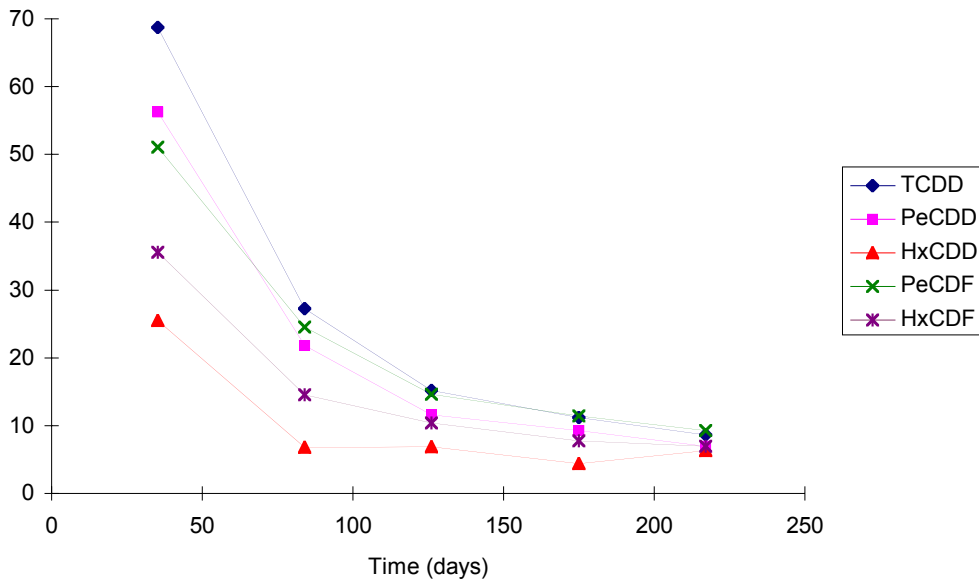


Figure 1: Concentration versus time for PCDD/Fs in subcutaneous fat of treated cattle

References

- Startin J.R., Wright C., Kelly M., Rose M. and Harrison N., 1994, Levels of PCDD and PCDF congeners in milk from farms near Walsley, U.K. *Organohalogen Compounds*, **21**, 151-154.
- Startin, N., Gem, M.G. de M., Startin J.R., Wright C., Kelly M. and Rose M., 1996, PCDDs and PCDFs in milk from farms in North Yorkshire, UK. *Chemosphere*, **32**, 453-460.
- Wong, M., Berende, P.L.M., Derks, H.J.G.M., Liem, A.R.D. Everts, H. and de Jong, A.P.J.M., 1991, De toxicokinetiek van PCDD's en PCDF's in niet lacterende koeien (vetweiders) RIVM report 328904003.
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- Wong, M., Hansson, M., Sjöström, M., Rappe, C., Kahn, P., Gochfeld, M., Velez, H., Ghent-Guenther, T., Wilson, W.P., 1988, Development and Validation of a Method for Determination of PCDDs and PCDFs in Human Blood Plasma. A Multivariate Comparison of Blood and Adipose Tissue Levels Between Viet Nam Veterans and Matched Controls. *Chemosphere*, **17**(9), 1663-1692.

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Results and discussion

Table 1 presents the concentrations of the five congeners in the different tissues analysed. All data are reported on a fat weight basis. In cases where more than one sample of a particular tissue was analysed, the figures used are the mean values.

The agreement between individual data points in these instances was similar to that obtained for the reference meat sample (see under quality control, below).

The concentrations of PCDD/Fs in control animals was low compared with the treated animals throughout the study. In the case of Group 1 dosed animals, slaughtered five weeks after the start of dosing, the two different depot fat stores (sub-cutaneous fat and perirenal fat) generally had similar concentrations to the value predicted from earlier studies.^{3,4} However, liver and muscle contained around 10 and 5 times higher concentrations respectively of PCDD/Fs (on a fat basis) than the fat stores. The differences had narrowed by the time that Groups 2 and 3 animals were slaughtered although liver and muscle concentrations were still typically twice those present in fat stores. Preferential accumulation in animal livers following high doses is in line with published results obtained in other species, but the higher concentrations found in muscle were not predicted. This fact suggested that the distribution phase was incomplete and that PCDD/Fs were still associated mainly with circulating blood lipids rather than as an equilibrium with depot fat.

Changes in PCDD/F concentration in subcutaneous fat with time, incorporating both post-mortem and biopsy data from different animals, are shown in Figure 1. The figures are corrected for weight gain of the animals during the study. Starting concentrations were less than 5 ng/kg for each congener in each animal. Each curve follows the same form, showing a large increase in concentration when sampled one week post-dosing, followed by a rapid decline over the first part of the depletion study with a slower fall in concentration thereafter.

Half-lives for each congener can be estimated from the plots as follows: 2,3,7,8-TCDD (93 days), 1,2,3,7,8-PeCDD (126 days), 1,2,3,6,7,8-HxCDD (148 days), 2,3,4,7,8-PeCDF (106 days) and 1,2,3,4,7,8-HxCDF (124 days). These values were broadly in line with calculated values in the literature.^{3,4} The high value for 1,2,3,6,7,8-HxCDD was affected by a single data point which was out of line with the general pattern observed.

Quality control

The relative standard deviation for the recovery of the five congeners ranged from 8 % - 23 % in the quality control sample.

Acknowledgement

This work was funded by the Joint Food Standards and Safety Group of the UK Ministry of Agriculture, Fisheries and Food; project FS2155.

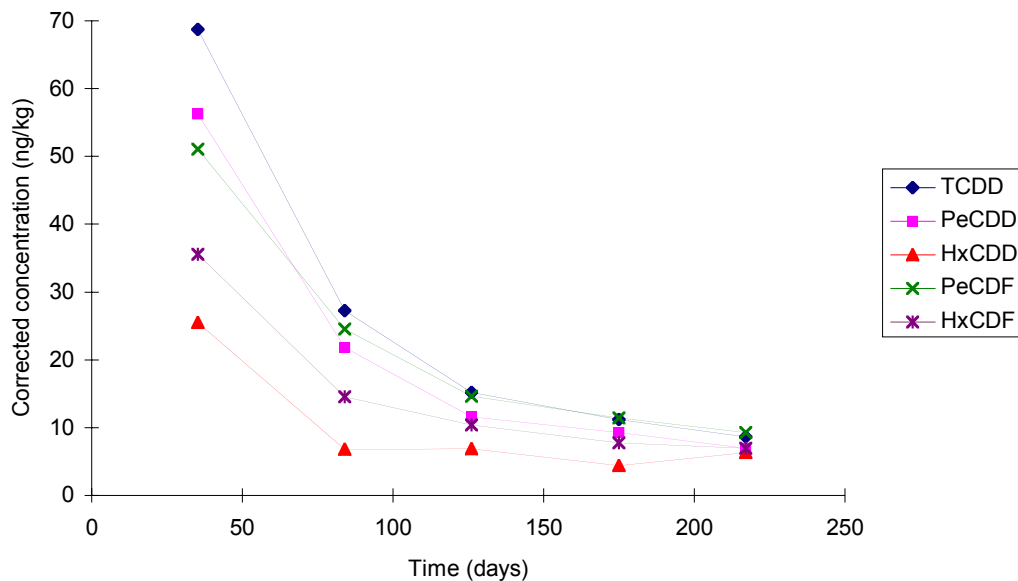


Figure 1: Concentration versus time for PCDD/Fs in subcutaneous fat of treated cattle

References

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5. Nygren, M., Hansson, M., Sjostrom, M., Rappe, C., Kahn, P., Gochfeld, M., Velez, H., Ghent-Guenther, T., Wilson, W.P., 1988, Development and Validation of a Method for Determination of PCDDs and PCDFs in Human Blood Plasma. A Multivariate Comparison of Blood and Adipose Tissue Levels Between Viet Nam Veterans and Matched Controls. *Chemosphere*, **17**(9), 1663-1692.

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Slaughter Time (weeks)	2,3,7,8-TCDD				1,2,3,7,8-PeCDD				1,2,3,6,7,8-HxCDD				2,3,4,7,8-PeCDF				1,2,3,4,7,8-HxCI		
	Tissue		Fat		Tissue		Fat		Tissue		Fat		Tissue		Fat		Tissue		
	L	M	S	P	L	M	S	P	L	M	S	P	L	M	S	P	L	M	
<u>Dosed animals</u>																			
1a	5	560	210	32	45	560	250	29	32	500	140	26	11	740	230	35	34	580	18
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2b	18	37	37	17	12	55	13	14	8.5	50	11	8.6	14	93	16	16	7.8	91	1
2c	18	30	21	15	15	75	21	12	11	74	18	9.5	12	120	20	16	17	140	2
3a	31	33	22	13	16	56	25	11	12	41	16	9.9	9.3	83	28	16	14	110	4
3b	31	16	38	13	16	39	44	11	12	31	20	9.3	12	64	31	14	15	77	3
3c	31	16	13	12	12	33	25	9.2	8.6	93	14	10	7.5	89	15	12	11	78	1
3d	31	58	N/A	13	12	92	N/A	11	8.6	60	N/A	8.3	12	120	N/A	13	11	140	N/A
<u>Control animals</u>																			
Group 0	-1 day	7.4	4.0	0.8	0.8	5.2	4.6	0.9	1.6	2.5	5.4	0.9	0.7	5.5	6.5	0.8	0.9	0.5	5
Group 1	5	6.1	2.6	1.1	1.0	3.2	2.2	1.0	0.6	2.2	2.1	1.0	0.6	3.7	2.9	0.6	0.7	2.6	3
Group 3	31	1.1	1.6	1.0	1.8	0.2	6.8	0.9	0.4	3.7	4.1	0.7	1.2	3.9	2.4	0.8	1.1	6.0	0
Group 3	31	2.7	0.8	0.7	2.0	0.2	7.3	0.7	3.3	0.2	4.9	0.6	2.9	0.3	0.4	0.6	0.8	5.2	0

N/A: Sample not available

L: Liver,

M: Muscle

S: Subcutaneous P: Peri-renal